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Biomedical Engineering

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# PCR purification of insert fragment

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**Title**
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# 1 TAE stock:

- Tris-base: 242 g
- Acetate (100% acetic acid): 57.1 ml
- EDTA: 100 ml 0.5M sodium EDTA
- Add Distilled H2O up to one litre.

To make 1x TAE from 50X TAE stock, dilute 20ml of stock into 980 ml of Distilled water

# 2 Agarose gel (0.8%):

- Measure out 0.8 g of agarose.
- Pour agarose powder into microwavable flask along with 100mL of 1xTAE (TBE is also possible)
- Microwave for 1-3min (until the agarose is completely dissolved and there is a nice rolling boil).
- Note: It is a good idea to microwave for 30-45sec, stop and swirl, and then continue towards a boil. Keep an eye on it as the initial boil has a tendency to boil over. Placing saran wrap over the top of the flask can help with this, but is not necessary if you pay close attention.
- Let agarose solution cool down for 5min, to 50C
- Add 10 uL SybrSafe (10,000x stock) and swirl
- Pour the agarose into a gel tray with the well comb (~20 uL per well) in place. Evt. merge pins of comb for larger well volume.
- Let sit at room temperature for 20-30 minutes, until it has completely solidified.

# 3 Loading Samples and Running an Agarose Gel:

- Add loading buffer to each of your digest samples. 6x Loading dye (Fermentas) in fridge in electrophoresis corner. Digested vector for gel extraction, insert and vector for imaging (load ~300 ng).
- Once solidified, remove tape and place the agarose gel into the gel box (electrophoresis unit).
- Fill gel box with 1xTAE (or TBE) until the gel is covered.
- Carefully load a molecular weight ladder into the first lane of the gel (in fridge in electrophoresis corner)
- Carefully load your samples into the additional wells of the gel.
- Run the gel at 100V until the dye line is approximately 75-80% of the way down the gel. Note: A typical run time is about 1-1.5 hours, depending on the gel concentration and voltage. Note: Black is negative, red is positive. (The DNA is negatively charged and will run towards the positive electrode.) Always Run to Red.
- Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.

# 4 DNA extraction from gel

- Place the gel on blue lamp. Use protecting glasses
- Cut out the band from the gel. Cut away all non-DNA containing gel parts. Transfer gel fragment to eppendorf tube
- Using a scale, weigh the tube with the gel fragment after zeroing the scale with an empty tube. Finally, you will want to isolate the DNA from the gel. This is most commonly done with a commercial gel purification kit, such as the QIAquick Gel Extraction Kit.

# 5 Protocol:

The QIAquick Gel Extraction Kit (cat. nos. 28704 and 28706) can be stored at room temperature (15–25°C) for up to 12 months.

**Remarks:**
- The yellow color of Buffer QG indicates a pH ≤7.5.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- Isopropanol (100%) and a heating block or water bath at 50°C are required.
- All centrifugation steps are carried out at 17,900 x g (13,000 rpm) in a
- conventional table-top microcentrifuge.

**Protocol:**
- Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.
- Weigh the gel slice in a colorless tube.
- Add 3 volumes Buffer QG to 1 volume gel (100 mg ~ 100 µl). For >2% agarose gels, add 6 volumes Buffer QG.
- Incubate at 50°C for 10 min (or until the gel slice has completely dissolved).
- Vortex the tube every 2–3 min to help dissolve gel.
- After the gel slice has dissolved completely, check that the color of the mixture is
- yellow (similar to Buffer QG without dissolved agarose).
- If the color of the mixture is orange or violet, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn yellow.
- Add 1 gel volume of isopropanol (2-propanol; stored in yellow, DUPA cabinet) to the sample and mix.
- Place a QIAquick spin column in a provided 2 ml collection tube or into a vacuum manifold.
- To bind DNA, apply the sample to the QIAquick column and centrifuge for 1 min or apply vacuum to the manifold until all the samples have passed through the column. Discard flow-through and place the QIAquick column back into the same tube. For sample volumes of >800 µl, load and spin/apply vacuum again.
- If the DNA will subsequently be used for sequencing, in vitro transcription, or microinjection, add 0.5 ml Buffer QG to the QIAquick column and centrifuge for 1 min or apply vacuum. Discard flow-through and place the QIAquick column back into the same tube.
- To wash, add 0.75 ml Buffer PE to QIAquick column and centrifuge for 1 min or apply vacuum.Discard flow-through and place the QIAquick column back into the same tube.
- Repeat the PE washing step another time
- Centrifuge the QIAquick column once more in the provided 2 ml collection tube for 1 min at 17,900 x g (13,000 rpm) to remove residual wash buffer.

- Place QIAquick column into a clean 1.5 ml microcentrifuge tube.
- To elute DNA, add 42 µl water to the center of the QIAquick membrane and centrifuge the column for 1min. Let the column stand for 1 min, and then centrifuge.

**Imaging of vector and insert band loaded on agarose gel**

# 6 DNA quantification:

- Before measuring any samples, be sure to ‘blank’ the spectrophotometer using the solution the DNA is resuspended in, but with no DNA added. 'Blanking' measures the background inherent to the machine and your solvent.
- If using a NanoDrop to measure your samples, place 2 µL of mini-prepped DNA onto the pedestal.
- Close the lid and click measure, be sure to record the concentration and purity.
  Note: Purity is measured under the 260/280 column (A good purity ranges from 1.80-2.00).
- Repeat for each sample.